



SEQUENCE LISTING

<110> Shyur, Lie-Fen
Chen, Jui-Lin
Yang, Ning-Sun

<120> A Truncated Form of Fibrobacter Succinogenes 1,3-1,4-Beta-D-Glucanase With Improved

Enzymatic Activity And Thermo-Tolerance

<130> 4910-8

<140> US 09/654,652

<141> 2000-09-05

<150>

<151>

<160> 6

<210> 1

<211> 248

<212> PRT

<213> Artificial Sequence

<220>

<223> Modified enzyme with enhanced activity and thermal stability

<400> 1

Met Val Ser Ala Lys Asp Phe Ser Gly Ala Glu Leu Tyr Thr Leu Glu Glu Val Gln Tyr

1	5	10	15	20
Gly	Lys	Phe	Glu	Ala
Arg	Met	Lys	Met	Ala
Ala	Ala	Ser	Gly	Thr
Val	Ser	Ser	Met	Phe
25	30	35	40	
Leu	Tyr	Gln	Asn	Gly
Ser	Glu	Ile	Ala	Asp
Gly	Arg	Pro	Trp	Val
Glu	Val	Asp	Ile	Glu
45	50	55	60	
Val	Leu	Gly	Lys	Asn
Pro	Gly	Ser	Phe	Gln
Ser	Asn	Ile	Ile	Thr
Gly	Lys	Ala	Gly	Ala
65	70	75	80	
Gln	Lys	Thr	Ser	Glu
Lys	His	His	Ala	Val
Ser	Pro	Ala	Ala	Asp
Gln	Ala	Phe	His	Thr
85	90	95	100	
Tyr	Gly	Leu	Glu	Trp
Thr	Pro	Asn	Tyr	Val
Arg	Trp	Thr	Val	Asp
Gly	Gln	Glu	Val	Arg
105	110	115	120	
Lys	Thr	Glu	Gly	Gln
Val	Ser	Asn	Leu	Thr
Gly	Thr	Gln	Gly	Leu
Arg	Phe	Asn	Leu	
125	130	135	140	
Trp	Ser	Ser	Glu	Ser
Ala	Ala	Trp	Val	Gly
Gln	Phe	Asp	Glu	Ser
Lys	Leu	Pro	Leu	Phe
145	150	155	160	
Gln	Phe	Ile	Asn	Trp
Val	Lys	Val	Tyr	Lys
Tyr	Thr	Pro	Gly	Gln
Gly	Glu	Gly	Gly	Ser
165	170	175	180	
Asp	Phe	Thr	Leu	Asp
Trp	Thr	Asp	Asn	Phe
Asp	Thr	Phe	Asp	Gly
Ser	Arg	Trp	Gly	Lys
185	190	195	200	
Gly	Asp	Trp	Thr	Phe
Asp	Gly	Asn	Arg	Val
Asp	Leu	Thr	Asp	Lys
Asn	Ile	Tyr	Ser	Arg
205	210	215	220	
Asp	Gly	Met	Leu	Ile
Leu	Ala	Leu	Thr	Arg
Lys	Gly	Gln	Glu	Ser
Phe	Asn	Gly	Gln	Val
225	230	235	240	
Pro	Arg	Asp	Asp	Glu
Pro	Ala	Pro		
245				

<210> 2

<211> 267

<212> PRT

<213> Artificial Sequence

<220>

<223> Modified enzyme with enhanced activity and thermal stability

<400> 2

Met	Val	Ser	Ala	Lys	Asp	Phe	Ser	Gly	Ala	Glu	Leu	Tyr	Thr	Leu	Glu	Glu	Val	Gln	Tyr	1	5	10	15	20
Gly	Lys	Phe	Glu	Ala	Arg	Met	Lys	Met	Ala	Ala	Ala	Ser	Gly	Thr	Val	Ser	Ser	Met	Phe	25	30	35	40	
Leu	Tyr	Gln	Asn	Gly	Ser	Glu	Ile	Ala	Asp	Gly	Arg	Pro	Trp	Val	Glu	Val	Asp	Ile	Glu	45	50	55	60	
Val	Leu	Gly	Lys	Asn	Pro	Gly	Ser	Phe	Gln	Ser	Asn	Ile	Ile	Thr	Gly	Lys	Ala	Gly	Ala	65	70	75	80	
Gln	Lys	Thr	Ser	Glu	Lys	His	His	Ala	Val	Ser	Pro	Ala	Ala	Asp	Gln	Ala	Phe	His	Thr	85	90	95	100	
Tyr	Gly	Leu	Glu	Trp	Thr	Pro	Asn	Tyr	Val	Arg	Trp	Thr	Val	Asp	Gly	Gln	Glu	Val	Arg	105	110	115	120	
Lys	Thr	Glu	Gly	Gly	Gln	Val	Ser	Asn	Leu	Thr	Gly	Thr	Gln	Gly	Leu	Arg	Phe	Asn	Leu	125	130	135	140	
Trp	Ser	Ser	Glu	Ser	Ala	Ala	Trp	Val	Gly	Gln	Phe	Asp	Glu	Ser	Lys	Leu	Pro	Leu	Phe	145	150	155	160	
Gln	Phe	Ile	Asn	Trp	Val	Lys	Val	Tyr	Lys	Tyr	Thr	Pro	Gly	Gln	Gly	Glu	Gly	Gly	Ser	165	170	175	180	
Asp	Phe	Thr	Leu	Asp	Trp	Thr	Asp	Asn	Phe	Asp	Thr	Phe	Asp	Gly	Ser	Arg	Trp	Gly	Lys	185	190	195	200	
Gly	Asp	Trp	Thr	Phe	Asp	Gly	Asn	Arg	Val	Asp	Leu	Thr	Asp	Lys	Asn	Ile	Tyr	Ser	Arg	205	210	215	220	
Asp	Gly	Met	Leu	Ile	Leu	Ala	Leu	Thr	Arg	Lys	Gly	Gln	Glu	Ser	Phe	Asn	Gly	Gln	Val	225	230	235	240	
Pro	Arg	Asp	Asp	Glu	Pro	Ala	Pro	Asn	Ser	Ser	Ser	Val	Asp	Lys	Leu	Ala	Ala	Ala	Leu	245	250	255	260	
Glu	His	His	His	His	His	His	His													265				

<210> 3

<211> 349

<212> PRT

<213> Fibrobacter succinogenes

<220>

<223>

<400> 3

Met	Asn	Ile	Lys	Lys	Thr	Ala	Val	Lys	Ser	Ala	Leu	Ala	Val	Ala	Ala	Ala	Ala	Ala	Ala	1	5	10	15	20
Leu	Thr	Thr	Asn	Val	Ser	Ala	Lys	Asp	Phe	Ser	Gly	Ala	Glu	Leu	Tyr	Thr	Leu	Glu	Glu	25	30	35	40	
Val	Gln	Tyr	Gly	Lys	Phe	Glu	Ala	Arg	Met	Lys	Met	Ala	Ala	Ala	Ser	Gly	Thr	Val	Ser	45	50	55	60	
Ser	Met	Phe	Leu	Tyr	Gln	Asn	Gly	Ser	Glu	Ile	Ala	Asp	Gly	Arg	Pro	Trp	Val	Glu	Val	65	70	75	80	
Asp	Ile	Glu	Val	Leu	Gly	Lys	Asn	Pro	Gly	Ser	Phe	Gln	Ser	Asn	Ile	Ile	Thr	Gly	Lys	85	90	95	100	
Ala	Gly	Ala	Gln	Lys	Thr	Ser	Glu	Lys	His	His	Ala	Val	Ser	Pro	Ala	Ala	Asp	Gln	Ala	105	110	115	120	
Phe	His	Thr	Tyr	Gly	Leu	Glu	Trp	Thr	Pro	Asn	Tyr	Val	Arg	Trp	Thr	Val	Asp	Gly	Gln	125	130	135	140	
Glu	Val	Arg	Lys	Thr	Glu	Gly	Gly	Gln	Val	Ser	Asn	Leu	Thr	Gly	Thr	Gln	Gly	Leu	Arg	145	150	155	160	
Phe	Asn	Leu	Trp	Ser	Ser	Glu	Ser	Ala	Ala	Trp	Val	Gly	Gln	Phe	Asp	Glu	Ser	Lys	Leu	165	170	175	180	
Pro	Leu	Phe	Gln	Phe	Ile	Asn	Trp	Val	Lys	Val	Tyr	Lys	Tyr	Thr	Pro	Gly	Gln	Gly	Glu	185	190	195	200	

Gly	Gly	Ser	Asp	Phe	Thr	Leu	Asp	Trp	Thr	Asp	Asn	Phe	Asp	Thr	Phe	Asp	Gly	Ser	Arg
				205					210					215					220
Trp	Gly	Lys	Gly	Asp	Trp	Thr	Phe	Asp	Gly	Asn	Arg	Val	Asp	Leu	Thr	Asp	Lys	Asn	Ile
				225					230					235					240
Tyr	Ser	Arg	Asp	Gly	Met	Leu	Ile	Leu	Ala	Leu	Thr	Arg	Lys	Gly	Gln	Glu	Ser	Phe	Asn
				245					250					255					260
Gly	Gln	Val	Pro	Arg	Asp	Asp	Glu	Pro	Ala	Pro	Gln	Ser	Ser	Ser	Ser	Ala	Pro	Ala	Ser
				265					270					275					280
Ser	Ser	Ser	Val	Pro	Ala	Ser	Ser	Ser	Ser	Val	Pro	Ala	Ser	Ser	Ser	Ser	Ala	Phe	Val
				285					290					295					300
Pro	Pro	Ser	Ser	Ser	Ser	Ala	Thr	Asn	Ala	Ile	His	Gly	Met	Arg	Thr	Thr	Pro	Ala	Val
				305					310					315					320
Ala	Lys	Glu	His	Arg	Asn	Leu	Val	Asn	Ala	Lys	Gly	Ala	Lys	Val	Asn	Pro	Asn	Gly	His
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Lys	Arg	Tyr	Arg	Val	Asn	Phe	Glu	His											
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<210> 4
 <211> 744
 <212> DNA
 <213> Artificial Sequence
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 <223> Code for a modified enzyme
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ctctaccaga	atggttccga	aatcgccgat	ggaaggccct	gggtagaagt	ggatattgaa	180
gttctcggca	agaatccggg	cagtttccag	tccaacatca	ttaccggtaa	ggccggcgca	240
caaaagacta	gcgaaaagca	ccatgctgtt	agccccgcgc	ccgatcaggc	tttccacacc	300
tacggtctcg	aatggactcc	gaattacgtc	cgctggactg	ttgacggtca	ggaagtccgc	360
aagacggaag	gtggccaggt	ttccaacttg	acaggtagac	agggactccg	ttttaacctt	420
tggtcgtctg	agagtgcggc	ttgggttggc	cagttcgtatg	aatcaaaagct	tccgcttttc	480
cagttcatca	actgggtcaa	ggtttataag	tatacgccgc	gccagggcga	aggcggcagc	540
gactttacgc	ttgactggac	cgacaatttt	gacacgtttg	atggctcccgc	ctggggcaag	600
ggtgactgga	catttgacgg	taaccgtgtc	gacctcaccg	acaagaacat	ctactccaga	660
gatggcatgt	tgatcctcgc	cctcaccgcg	aaaggtcagg	aaagcttcaa	cggccagggtt	720
ccgagagatg	acgaacctgc	tccg				744

<210> 5
 <211> 804
 <212> DNA
 <213> Artificial Sequence
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 <223> Code for a modified enzyme
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ctctaccaga	atggttccga	aatcgccgat	ggaaggccct	gggtagaagt	ggatattgaa	180
gttctcggca	agaatccggg	cagtttccag	tccaacatca	ttaccggtaa	ggccggcgca	240
caaaagacta	gcgaaaagca	ccatgctgtt	agccccgcgc	ccgatcaggc	tttccacacc	300
tacggtctcg	aatggactcc	gaattacgtc	cgctggactg	ttgacggtca	ggaagtccgc	360
aagacggaag	gtggccaggt	ttccaacttg	acaggtagac	agggactccg	ttttaacctt	420
tggtcgtctg	agagtgcggc	ttgggttggc	cagttcgtatg	aatcaaaagct	tccgcttttc	480
cagttcatca	actgggtcaa	ggtttataag	tatacgccgc	gccagggcga	aggcggcagc	540
gactttacgc	ttgactggac	cgacaatttt	gacacgtttg	atggctcccgc	ctggggcaag	600
ggtgactgga	catttgacgg	taaccgtgtc	gacctcaccg	acaagaacat	ctactccaga	660
gatggcatgt	tgatcctcgc	cctcaccgcg	aaaggtcagg	aaagcttcaa	cggccagggtt	720

ccgagagatg acgaacctgc tccgaattcg agctccgtcg acaagcttgc ggccgcactc 780
gagcaccacc accaccacca ctga 804

<210> 6
<211> 1050
<212> DNA
<213> Fibrobacter succinogenes
<220>
<223>
<400> 6

atgaacatca	agaaaactgc	agtcaagagc	gctctcgccg	tagcagccgc	agcagcagcc	60
ctcaccacca	atgttagcgc	aaaggatttt	agcggtgccg	aactctacac	gttagaagaa	120
gttcagtacg	gtaagtttga	agcccgtatg	aagatggcag	ccgcatcggg	aacagtcagt	180
tccatgttcc	tctaccagaa	tggttccgaa	atcgccgatg	gaaggccctg	ggtagaagtg	240
gatatagaag	ttctcggcaa	gaatccgggc	agtttccagt	ccaacatcat	taccggtaag	300
gccggcgcac	aaaagactag	cgaaaagcac	catgctgtta	gccccgcgcg	cgatcaggct	360
ttccacacct	acgggtctcg	atggactccg	aattacgtcc	gctggactgt	tgacggtcag	420
gaagtccgca	agacggaagg	tggccagggt	tccaacttga	caggtaacaca	gggactccgt	480
tttaaccttt	ggtcgtctga	gagtgcggct	tgggttgccc	agttcgatga	atcaaagctt	540
ccgcttttcc	agttcatcaa	ctgggtcaag	gtttataagt	atacgccggg	ccaggggcgaa	600
ggcggcagcg	actttacgct	tgactggacc	gacaattttg	acacgtttga	tggtccccgc	660
tggggcaagg	gtgactggac	atgtgacggt	aaccgtgtcg	acctcaccga	caagaacatc	720
tactccagag	atggcatggt	gacccctgcc	ctcaccgcga	aaggtcagga	aagcttcaac	780
ggccagggtc	cgagagatga	cgaacctgct	ccgcaatctt	ctagcagcgc	tccggcatct	840
tctagcagtg	ttccggcaag	ctcctctagc	gtccctgcct	cctcgagcag	cgatttggtt	900
ccgccgagct	cctcgagcgc	cacaaacgca	atccacggaa	tgcgcacaa	tccggcagtt	960
gcaaagggaac	accgcaatct	cgtgaacgcc	aagggtgcc	aggtgaacc	gaatggccac	1020
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<210> 7
<211> 13
<212> DNA
<213> Artificial Sequence
<220>
<223> PCR primer
<400> 7

tcaccaccat ggttagcgca aag 13

<210> 8
<211> 15
<212> DNA
<213> Artificial Sequence
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<223> PCR primer
<400> 8

gccacgaatt ctgttcaaag ttcac 15

<210> 9
<211> 17
<212> DNA
<213> Artificial Sequence
<220>
<223> PCR Primer
<400> 9

cagccggcga tggccatggt tagcgca 17

<210> 10
<211> 19
<212> DNA
<213> Artificial Sequence
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<223> PCR Primer
<400> 10
ctgctagaag aattcggagc aggttcgtc

19
